## Symptoms of prenatal depression are associated with raised salivary alpha-amylase levels

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Running Title: Prenatal depression and salivary alpha-amylase

## Abstract

Purpose

Prenatal depression increases risk for a number of adverse offspring outcomes, however the biological mechanisms underlying this association remain unclear. It has been suggested that maternal glucocorticoids may mediate this link, though supporting evidence has been mixed. An alternative mechanism of effect may be via depression-induced changes in maternal sympathetic nervous system (SNS) function. We examined this hypothesis by determining the relationship between symptoms of maternal prenatal depression and diurnal salivary alpha-amylase (sAA) levels.

Methods

76 pregnant women were recruited during either the second or third trimester of pregnancy. Participants self-reported depressive symptoms using the Edinburgh Postnatal Depression Scale. Saliva samples, to be assayed for alpha-amylase activity, were collected at home over two working days.

Results

Participants with depressive symptoms in later pregnancy, had elevated awakening sAA levels compared with non-depressed controls (t(73)=-2.737, p=0.008), and continued to have raised sAA throughout the day (F(1)=10.924, p=0.002).

Conclusions

Our findings highlight that symptoms of depression during late pregnancy are associated with increased maternal SNS activity. Thus, changes in maternal SNS function, which may include increased vasoconstriction and reduced fetal blood flow, could, in part, mediate associations between prenatal depression and adverse offspring outcomes.

Keywords: prenatal depression, alpha-amylase, sympathetic nervous system

# Introduction

Prenatal psychological distress, which encompasses feelings of depression, anxiety and stress, increases risk for adverse offspring physical and psychological health outcomes. For example, prenatally distressed women are at greater risk of both preterm birth ([Class et al., 2011](#_ENREF_13); [Copper et al., 1996](#_ENREF_14); [Nkansah-Amankra et al., 2010](#_ENREF_44)) and of having a low birth weight baby ([Sable and Wilkinson, 2000](#_ENREF_56); [Zhu et al., 2010](#_ENREF_73)), which increases risk for offspring diabetes ([Whincup et al., 2008](#_ENREF_71)) and cardiovascular disease ([Barker, 1999](#_ENREF_3); [Huxley et al., 2007](#_ENREF_29)) in later life. Further, exposure to maternal psychological distress has been linked with increased rates of behavioural and emotional problems in childhood ([O'Connor et al., 2002b](#_ENREF_46); [O'Connor et al., 2003](#_ENREF_47)), and increased rates of psychiatric disease in adulthood ([Pearson et al., 2013a](#_ENREF_50); [Van den Bergh and Marcoen, 2004](#_ENREF_68); [Van den Bergh et al., 2008](#_ENREF_69)). Interestingly, these effects appear to be independent of postnatal mood ([O'Connor et al., 2002a](#_ENREF_45); [Pearson et al., 2013a](#_ENREF_50)), and there is direct evidence to suggest that developmental changes in these offspring may begin during the prenatal period. For example, fetuses of prenatally distressed mothers show perturbed fetal heart rate responses to both maternal stress ([Monk et al., 2000](#_ENREF_35)) and direct stimulation ([Fernandes et al., 2014](#_ENREF_20)), and are also more active ([Dieter et al., 2008](#_ENREF_17)), compared with fetuses of non-stressed mothers. However, despite extensive efforts, the *in utero* biological processes that may link prenatal disturbed mood with altered offspring development remain unclear.

In recent years, the field of perinatal psychiatry has been largely focused on alterations of the maternal and fetal hypothalamic-pituitary adrenal (HPA) axes as a potential biological mechanism that may mediate the link between prenatal disturbed mood and perturbed fetal and infant development ([Braithwaite et al., 2014](#_ENREF_9); [Glover, 2011](#_ENREF_25)). This is largely because in non-pregnant populations symptoms of depression are associated with cortisol hyper-secretion ([Bhagwagar et al., 2005](#_ENREF_5); [Cowen, 2002](#_ENREF_15); [Herbert, 2013](#_ENREF_28)). However, data linking symptoms of mood disturbance in pregnancy with changes in maternal HPA function have been mixed, with evidence both for ([Giesbrecht et al., 2012](#_ENREF_23); [Murphy et al., 2014](#_ENREF_39); [O'Connor et al., 2013](#_ENREF_48); [Obel et al., 2005](#_ENREF_49)) and against ([Hellgren et al., 2013](#_ENREF_27); [Pluess et al., 2012](#_ENREF_52)) cortisol hyper-secretion. One possible explanation for the disparate findings is that cortisol levels rise throughout pregnancy, regardless of mood state, due to the release of corticotrophin-releasing hormone (CRH) from the placenta. By the end of gestation, maternal serum cortisol levels are 4 times higher than in the non-pregnancy state ([Lindsay and Nieman, 2005](#_ENREF_33)) and therefore detecting mood-induced changes in HPA function becomes difficult. It is possible that levels of placental CRH may be important in the onset of depressive symptoms, as raised placental CRH has been associated with both prenatal ([Rich-Edwards et al., 2008](#_ENREF_53)) and postnatal depressive symptoms ([Yim et al., 2009](#_ENREF_72)). However, an alternative explanation is that maternal glucocorticoids may not be as central in mediating associations between prenatal mood and fetal developmental trajectories as previously thought.

Changes in maternal sympathetic nervous system (SNS) activity have been proposed as an alternative pathway by which disturbances in prenatal mood may impact fetal development ([Braithwaite et al., 2014](#_ENREF_9); [Talge et al., 2007](#_ENREF_64)). As well as the HPA axis, psychological distress also activates the SNS, which results in an increase in the circulating levels of noradrenaline. Evidence for the involvement of noradrenaline in depression is abundant, for example a number of antidepressants inhibit noradrenaline reuptake, and recent studies on neuronal pathways and depressive symptoms highlight the specific role of noradrenaline in this disorder, for review see [Moret and Briley (2011)](#_ENREF_37). In pregnancy, noradrenaline does not directly cross the placenta ([Giannakoulopoulos et al., 1999](#_ENREF_22)); however its release may indirectly affect the fetus by initiating vasoconstriction and disrupting uterine blood flow. The reduced or fluctuating transmission of oxygen to the fetus may perturb fetal growth, and increase risk for low birth weight or premature birth, which, as mentioned above, increases risk for poor offspring physical health ([Barker, 1999](#_ENREF_3); [Whincup et al., 2008](#_ENREF_71)). Further, disruption to normal brain development resulting from the lack of oxygen or nutrients may increase the likelihood of later psychological difficulties ([Morsing et al., 2011](#_ENREF_38)). In support of this hypothesis, animal research has shown that both acute stress and intravenous infusions of noradrenaline induce a decrease in uterine blood flow ([Shnider et al., 1979](#_ENREF_57); [Stevens and Lumbers, 1995](#_ENREF_60)). Initial human investigations mirrored the animal findings, and reported associations between prenatal anxiety and decreased uterine blood flow ([Sjostrom et al., 1997](#_ENREF_58); [Teixeira et al., 1999](#_ENREF_66)). However notably, there have been a number of failed replication attempts ([Harville et al., 2008](#_ENREF_26); [Kent et al., 2002](#_ENREF_32); [Mendelson et al., 2011](#_ENREF_34); [Monk et al., 2012](#_ENREF_36)). Discordance in the published literature may be attributable to methodological differences in the existing studies, or because doppler assessments of uterine blood flow are difficult to administer during pregnancy in a controlled laboratory setting.

An alternative method for assessing maternal SNS function may be via the use of the salivary biomarker, alpha-amylase. In recent years, salivary alpha-amylase (sAA) has been proposed as a sensitive biomarker of stress related changes in SNS activity, and there is a growing body of literature to support this idea ([Engert et al., 2011](#_ENREF_18); [Nater and Rohleder, 2009](#_ENREF_41); [Nater et al., 2007](#_ENREF_42); [Rohleder and Nater, 2009](#_ENREF_54)). Alpha-amylase is an enzyme produced by the salivary glands, which is involved in the initiation of starch break-down in the oral cavity ([Nater and Rohleder, 2009](#_ENREF_41)). Production of sAA is controlled by SNS innervation, therefore increased sAA concentrations are expected during periods of psychological distress, when activation of the SNS is high. Indeed, a number of studies in non-pregnant populations have found evidence to support this ([Bosch et al., 1996](#_ENREF_6); [Bosch et al., 2003](#_ENREF_7); [Chatterton et al., 1997](#_ENREF_12); [Skosnik et al., 2000](#_ENREF_59); [Takai et al., 2004](#_ENREF_62), [2007](#_ENREF_63)). Increased sAA in response to acute stress has also been associated with the expected increase in circulating noradrenaline ([Rohleder et al., 2004](#_ENREF_55); [Thoma et al., 2012](#_ENREF_67)). Further, sAA levels have also been reported to be inflated in people with depression ([Ishitobi et al., 2010](#_ENREF_31); [Tanaka et al., 2012](#_ENREF_65); [Veen et al., 2013](#_ENREF_70)).

Studies of sAA in pregnancy are limited, however increases in sAA concentration in response to an acute stressor have been reported in a population of pregnant participants, during both the second and third trimester ([Nierop et al., 2006](#_ENREF_43)). Just one study has assessed the effects of mood disturbance during pregnancy on diurnal sAA, and found a significant association between chronic symptoms of anxiety and increased diurnal levels of sAA ([Giesbrecht et al., 2013](#_ENREF_24)), however symptoms of depression and positive mood were not associated with diurnal sAA. On the other hand, momentary depression was associated with raised sAA whereas momentary anxiety was not. This initial finding, that prenatal mood disturbance is associated with raised sAA, requires replication in a different cohort, and mood-associated changes in sAA concentrations across gestation are yet to be fully characterised. Furthermore, it is currently unclear whether clinically relevant symptoms of depression are associated with increased sAA during pregnancy. Thus, the aim of this study is to investigate whether prenatal depression may be associated with altered maternal sAA levels in the second and third trimester.

# Materials and methods

## Participants

The participants (N=76) were derived from a larger cohort of pregnant women participating in a longitudinal study based in Oxford, UK, designed to investigate the effects of prenatal mood disturbance on maternal and infant stress responses. Inclusion criteria for pregnant participants were as follows: 18 years of age or over, primiparous, a singleton pregnancy (i.e. not pregnant with twins or triplets), not currently taking any steroid-based medication or anti-depressants, no medical complications associated with the pregnancy. The study was reviewed and approved by the South Central Oxford B Research Ethics Committee (REF: 12/SC/0473), and all participants provided informed consent.

## Procedure

Participants attended a test session during either the second or third trimester of pregnancy, which took place either at the Department of Psychiatry, University of Oxford, or at the participant’s home. Participants self-reported symptoms of depression using a paper-based questionnaire (Edinburgh Postnatal Depression Scale). Participants were also required to provide 5 saliva samples using the passive drool method, and the researcher instructed the participant on how to collect the saliva samples using 2ml cryovials and saliva collection aids (Salimetrics, UK). Participants were then asked to collect a further six saliva samples at home over two working days (3 per day), and were provided with a pack of six 2ml cryovials and six saliva collection aids, as well as a stamped-addressed envelope to return the samples. On each day, samples were collected immediately after awakening, 30 minutes and 12 hours post-awakening. Participants stored the samples in their home fridges at 4**°**C, before returning them to the Department of Psychiatry. Samples were shipped at room temperature, and remained at room temperature for a maximum of 24 hours before being frozen at -20**°**C at the Department of Psychiatry on arrival. Previous research has shown that salivary alpha-amylase samples remain stable for up to 4 days at room temperature, therefore the samples used in this study remain valid and stable.

## Measures

*Edinburgh Postnatal Depression Scale (EPDS)*. The EPDS is the most widely used self-report questionnaire to identify symptoms of depression during the peripartum period. The scale consists of 10 items that describe common symptoms of depression, and each item is scored from 0 to 3, with a maximum score of 30. A cut-off score of 10 is frequently used to identify a group ‘at risk’ of depression ([Adewuya et al., 2006](#_ENREF_1); [Adouard et al., 2005](#_ENREF_2); [Bergink et al., 2011](#_ENREF_4); [Felice et al., 2004](#_ENREF_19); [Murray and Cox, 1990](#_ENREF_40)). A recent study has shown that using a cut-off of 10 in the second and third trimester of pregnancy provides a good balance between sensitivity (70-79%) and specificity (96-97%) ([Bergink et al., 2011](#_ENREF_4)).

*Salivary alpha-amylase*. Salivary alpha-amylase kits were sourced from Salimetrics, UK. The salivary alpha-amylase test determines the amount of alpha-amylase activity present in each sample. It works by using the chromogenic substance 2-chloro-p-nitrophenol linked with maltotriose. The alpha-amylase present in saliva breaks down this substance and yields 2-chloro-p-nitrophenol, which is yellow and can be measured spectrophotometrically at 405nm. The amount of alpha-amylase present in the sample is directly proportional to the increase in absorbance at 405nm. The protocol used here differs from that recommended by the manufacturer in two ways; firstly, the volumes added to the 96-well plate were reduced to prevent sample loss during the mixing phase, but the ratio of substrate to sample was maintained at that recommended by the manufacturer (40:1). Secondly, the method of calculation of the alpha-amylase activity was changed from the manufacturer’s recommendation of using their pre-prepared equation. This was partly as a consequence of changing the sample volume, which would alter the path length from that given in the equation, but also the equation does not take into account test to test variability due to age of substrate and other experimental errors. Therefore, the amount of amylase activity was calculated using a six point standard curve (one per plate) made from a serial dilution of the high control provided by the manufacturer (with a concentration 285 units/ml +/-71). To reduce plate-to-plate variation, all reagents were from the same lot and were pooled before use; the same standard curve was used throughout the experiment.

Briefly the protocol was as follows: Saliva samples were diluted 1:200 in assay diluent. 5ul of diluted saliva was added to a well of a 96 well plate (16 wells were run at a time). 200ul of pre-warmed 37oC substrate solution (2-chloro-p-nitrophenol linked with maltotriose) was added with a multichannel pipette. The plate was immediately transferred to a heated spectrophotometer and mixed. Optical density readings were taken at 1 and 3 minutes and the change in optical density was calculated by subtracting the first from the second reading. Amount of amylase activity present in the samples was calculated using the standard curve. Following optimisation of the assay, this method was tested for reliability and found to be highly replicable (intra-assay coefficient of variance=3.73, inter-assay coefficient of variance=9.55).

The area under the curve (AUC) was calculated using the mean of the two awakening, 30 minutes and 12 hours post-awakening measures, and the log AUC was used as an outcome variable in these analyses.

## Statistical analyses

For analysis, participants were split into four groups based on their prenatal EPDS score and trimester. Those scoring 10 or above on the EPDS comprised the depressive-symptom group, whereas those who scored 9 or below on the EPDS comprised the control group (second trimester control group n=32, second trimester depressive-symptom group n=9, third trimester control group n=26, third trimester depressive-symptom group n=9). T-tests and Chi squared tests were used to compare the demographic characteristics of the two groups, and Pearson’s bivariate correlations were used to explore associations between variables. A repeated measures ANOVA was used to examine the change in salivary alpha-amylase concentrations over the course of the day with group (control *vs* depressive-symptom) and trimester (second *vs* third) as the between subjects factors. Finally, the log AUC of diurnal sAA measures was used as an index of total sAA levels, and groups were compared using a univariate ANOVA with trimester as a covariate.

# Results

## Sample characteristics

The demographic characteristics of this sample are presented in **Table 1**. All mothers in this study were primiparous; average age was 31.67 (SD 4.39). This primarily Caucasian (90.8%) group of women were highly educated (53.9% had a postgraduate degree). No participants reported smoking cigarettes during pregnancy, however 17.1% reported consuming 1-5 units of alcohol per week. 85.5% of participants reported that their pregnancy had been planned, and 26.3% reported a previous history of mental health disorders. In the main there were no significant differences in the demographic characteristics of the four groups (all p’s>0.05). However, the groups differed on the measure of prenatal depression, with participants of the depressive-symptom groups scoring significantly higher than participants of the control groups (**Table 1**: second trimester (F(1)=68.05, p<0.001), third trimester (F(1)=80.70, p<0.001)). Also, women of the depressive-symptom group were more likely to have a previous history of mental health disorders (**Table 1**: (*X*2(1)=10.40, p<0.05)). This effect was also significant among the third trimester participants when tested alone (**Table 1**: (*X*2(1)=2.51, p<0.05)), but not among second trimester participants.

None of the demographic variables were significantly correlated with each other. However, prenatal depression was significantly and positively correlated with awakening sAA (r=0.370, p=0.001). Also, all measures of sAA, and the log AUC, were significantly and positively correlated with each other (all p’s<0.01). Mean sAA measures and standard deviations are presented in **Table 2**.

## Diurnal salivary alpha-amylase

A repeated measures ANOVA was used to investigate the change in salivary alpha-amylase concentrations across the day. There was a significant within-subjects effect of time (F(2)=32.24, p<0.001), which reflected a decrease in salivary alpha-amylase concentrations from awakening to 30 minutes post-awakening (t(74)=6.289, p<0.001), and an increase in concentrations from 30 minutes post-awakening to 12 hours post-awakening (t(75)=-8.802, p<0.001), see **Figure 1**. There was also a significant interaction between time and prenatal depression (F(2)=4.615, p=0.011). Further investigation revealed that on awakening, participants with depressive symptoms had significantly higher alpha-amylase concentrations than the control group (t(73)=-2.737, p=0.008), however at 30 minutes and 12 hours post-awakening there was no effect of prenatal depression (p’s>0.05).

The between-subjects effect of trimester was non-significant (F(1)=0.258, p=0.613), the effect of prenatal depression tended towards significance (F(1)=3.220, p=0.077), and there was a significant interaction between prenatal depression and trimester (F(1)=4.046, p=0.040). To further investigate this interaction effect, second and third trimester participants were considered separately. In the third trimester, there was a significant effect of prenatal depression, such that those participants with symptoms of depression had greater salivary alpha-amylase levels across the three time points (see **Figure 1**: F(1)=10.924, p=0.002). However, in the second trimester there were no effects of prenatal depression on diurnal sAA (see **Figure 1**: F(1)=0.019, p=0.892).

## Log AUC salivary alpha-amylase

As an index of total alpha-amylase activity across the day, group differences in the log AUC were assessed using a univariate ANOVA. There was no significant effect of prenatal depression (F(1)=2.660, p=0.107) or trimester (F(1)=0.473, p=0.494), however the prenatal depression\*trimester interaction approached significance (F(1)=3.935, p=0.051). Further investigation revealed that for the third trimester participants, those with symptoms of depression had significantly higher log AUC alpha-amylase than control participants (see **Figure 2**: F(1)=6.912, p=0.013), but there was no effect of prenatal depression on the log AUC for the second trimester participants (see **Figure 2**: F(1)=0.060, p=0.808).

# Discussion

Exposure to prenatal mood disturbance, such as depressive symptoms, is predictive of adverse psychiatric and physical outcomes in offspring ([Buitelaar et al., 2003](#_ENREF_10); [Buss et al., 2010](#_ENREF_11); [Pearson et al., 2013b](#_ENREF_51); [Straub et al., 2012](#_ENREF_61)), and it has been proposed that elevated maternal glucocorticoids mediate these effects. However, disparity in the literature regarding prenatal mood and HPA function has propelled interest in other potential biological pathways, which may mediate the link between prenatal mood and adverse offspring development. In the current study, we report that symptoms of depression in late pregnancy are associated with raised awakening sAA levels, continue to be raised throughout the day. Thus, prenatal depression appears to be associated with altered SNS activity, which could have implications for fetal development and health outcomes. This is because increased SNS may be associated with increased vasoconstriction and reduced blood flow to the fetus, potentially resulting in intra-uterine growth restriction, preterm birth, and a low birth weight.

It should be noted, however, that there have been concerns in the literature that sAA is not a reliable marker of SNS function. This is because parasympathetic innervation of the salivary glands has also been shown to play a significant role in sAA release, via effects on salivary flow rate ([Bosch et al., 2011](#_ENREF_8)). Further, emotional valence, arousal and distress are all related to the interplay of the ANS, monoaminergic and endocrine systems ([Ganzel et al., 2010](#_ENREF_21)). Nonetheless, there is evidence to suggest that sAA is reflective of central noradrenergic function ([Cubala and Landowski, 2014](#_ENREF_16); [Veen et al., 2013](#_ENREF_70)), and despite concerns regarding the stimulation and control of sAA release, there is a general consensus in the existing literature that sAA is a measure of ANS sympathetic activity ([Engert et al., 2011](#_ENREF_18); [Nater and Rohleder, 2009](#_ENREF_41); [Nater et al., 2007](#_ENREF_42); [Rohleder and Nater, 2009](#_ENREF_54)).

Previous research in non-pregnant participants has reported significant associations between depressive symptoms and increased sAA concentrations ([Ishitobi et al., 2010](#_ENREF_31); [Tanaka et al., 2012](#_ENREF_65); [Veen et al., 2013](#_ENREF_70)), however such associations appear to be stronger in unremitted ([Ishitobi et al., 2010](#_ENREF_31)) and female patients ([Tanaka et al., 2012](#_ENREF_65)). Further, sAA levels have been related to antidepressant use; there is evidence to suggest that those patients using tri-cyclic antidepressant medication have higher sAA levels than those who use selective serotonin re-uptake inhibitors ([Veen et al., 2013](#_ENREF_70)). Apart from the aforementioned studies very little is known about sAA activity in un-medicated depressed subjects in general. Although these initial findings suggest that depressive symptoms may be associated with raised sAA, more research is required to fully characterise this relationship.

In the current study pregnant participants used the passive drool method to collect saliva samples, and the results presented here suggest that prenatal depression in late, but not mid, pregnancy is associated with raised sAA levels. Although [Giesbrecht et al. (2013)](#_ENREF_24) did not find that depressive symptoms were associated with raised sAA in pregnancy, they did report an association between anxiety and increases in sAA. When considering the effects of depression and anxiety on fetal development, these two psychological disorders are often combined under the umbrella of ‘psycholgical distress’, because of the large overlap in symptomatology and proposed biological mechanisms by which risk is transferred to the fetus. Thus, the novel finding of an association between depression and raised sAA is particularly pertinent when considering fetal development and offspring outcomes, because increased vasoconstriction may have implications for reduced fetal blood flow, growth and development. Thus, increased SNS activity could potentially explain why a number of observational studies have reported associations between prenatal mood disturbance and low birth weight/preterm infants ([Class et al., 2011](#_ENREF_13); [Copper et al., 1996](#_ENREF_14); [Nkansah-Amankra et al., 2010](#_ENREF_44); [Sable and Wilkinson, 2000](#_ENREF_56); [Zhu et al., 2010](#_ENREF_73)). However, it should be noted that direct studies of mood disturbance and uterine artery resistance have, in the main, failed to identify significant associations between mood disturbance and decreased fetal blood flow ([Harville et al., 2008](#_ENREF_26); [Kent et al., 2002](#_ENREF_32); [Mendelson et al., 2011](#_ENREF_34); [Monk et al., 2012](#_ENREF_36)). Therefore, it could be that sAA and Doppler assessments of fetal blood flow may measure different aspects of SNS activity, and sAA may not be a reliably associated with fetal blood flow. However, further assessments of maternal mood, sAA concentrations and uterine blood flow in the same cohort of pregnant participants are required to fully understand the relationship between the three variables.

Although there was a main effect of prenatal depression on awakening sAA, the results suggest that this effect was primarily driven by the third trimester group. Further, depression-associated increases in diurnal sAA were only evident in later pregnancy. The lack of effect of depression on diurnal sAA in the second trimester may be attributable to the small size of the depressive-symptom sub-group, resulting in reduced power to detect significant effects. A further explanation may be that previous studies have reported decreases in sAA from the second to third trimester ([Giesbrecht et al., 2013](#_ENREF_24); [Nierop et al., 2006](#_ENREF_43)), therefore the lower baseline sAA in later pregnancy may make mood associated increases in sAA easier to detect. In the current study we did not find a significant decrease in sAA from the second to third trimester, however Figure 1 shows that the mean sAA concentration of the third trimester control group is lower than the second trimester control group. Again, the lack of significant effect of trimester may be due to the small sample size or the confounding effects of depressive symptoms. On the other hand, throughout gestation maternal blood volume increases by 45%, and blood pressure decreases due to vasodilation caused by elevated progesterone ([Hytten, 1985](#_ENREF_30)). Thus, depression-associated changes in SNS function may be particularly prominent in later pregnancy, when maternal cardiac function is at its most modified state.

This study has a number of key methodological strengths. For example, the use of a well-validated and widely used self-report measure of prenatal depression is a significant strength, as is the highly replicable method for quantifying salivary alpha-amylase activity. Further, this sample of participants was un-medicated, and therefore the use of antidepressant medication did not influence or confound our findings. However, there are a number of limitations that require consideration. Firstly, a particularly striking characteristic of the sAA concentrations in our study is that the range appears to be very large (5.8–369.7 Units/ml), which reduces power to detect significant group differences. Indeed, this range is greater than the previous study to report on diurnal sAA in pregnancy (10.19–45.87 Units/ml) ([Giesbrecht et al., 2013](#_ENREF_24)). However, in a non-pregnant population, a range of 6–235 Units/ml has been reported across the day ([Nater et al., 2007](#_ENREF_42)), which is more similar to the concentrations reported here. It should also be noted, however, that the margin of error for the high standard supplied by Salimetrics UK, used to create a standard curve from which sAA concentrations were calculated, is +/- 71. This means that direct numerical comparisons to studies using different lots of high standards is difficult, however the main focus of the current study was to assess the difference between the groups, and not the absolute values. A further limitation is the small sample size and that that this group of participants were not a clinical sample with moderate to severe symptoms of depression, but instead were drawn from a community sample. As such, it was not possible to test for effects of severe depressive symptoms. Finally, three saliva samples were taken across each of two days in order to assess diurnal alpha-amylase. A more accurate method to index diurnal measures, and indeed to calculate the area under the curve, would have been to include more than three sampling times on each day.

# Conclusions

We found that symptoms of prenatal depression in late pregnancy are associated with raised awakening and diurnal sAA levels. Prenatal depression is associated with a number of adverse physical and psychological offspring outcomes; however the *in utero* biological mechanisms by which prenatal risk is transferred to fetal development are unclear. The results presented here suggest that one possible pathway of effect may be via depression-induced changes in maternal SNS function, which could potentially have short-term effects on fetal development, and long-term implications for offspring health.

# Tables

Table 1 Demographic characteristics of the sample

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Second trimester** | | **Third trimester** | |
| **Demographic Variables** | Control group (n=32) | Depressive symptom group (n=9) | Control group (n=26) | Depressive symptom group (n=9) |
| Age (m, SD) | 31.53, 5.13 | 31.33, 3.16 | 32.04, 4.10 | 31.44, 3.94 |
| Education (n,%) |  |  |  |  |
| GCSE/O-level | 1 (3.1) | - | - | - |
| A-level | 1 (3.1) | 1 (11.1) | 1 (3.8) | 1 (11.1) |
| Undergraduate degree | 12 (37.5) | 5 (55.6) | 10 (38.5) | - |
| NVQ | 1 (3.1) | - | - | 2 (22.2) |
| Postgraduate degree | 17 (53.1) | 3 (33.3) | 15 (57.7) | 6 (66.7) |
| Ethnicity (n,%) |  |  |  |  |
| Caucasian | 30 (93.8) | 7 (77.8) | 26 (100) | 7 (66.7) |
| Black |  |  |  | 1 (11.1) |
| Asian | 1 (3.1) | 1 (11.1) | - | - |
| Chinese | - | 1 (11.1) | - | 1 (11.1) |
| Mixed Race | 1 (3.1) | - | - | - |
| Alcohol units/week (n,%) |  |  |  |  |
| None | 27 (84.4) | 6 (66.7) | 23 (88.5) | 7 (77.8) |
| 1-5 | 5 (15.6) | 3 (33.3) | 3 (11.5) | 2 (22.2) |
| Cigarettes/week (n,%) |  |  |  |  |
| None | 24 (75) | 9 (100) | 23 (88.5) | 6 (66.7) |
| Did not respond | 8 (25) | - | 3 (11.5) | 3 (33.3) |
| Weeks of gestation (m, range) | 20.17  (14.6-26.6) | 20.70  (15.3-26.4) | 32.49  (27.1-39.3) | 35.32  (28.6-39.3) |
| Planned Pregnancy (n, %) | 28 (87.5) | 9 (100) | 21 (80.8) | 7 (77.8) |
| Previous history of mental health problems (n,%) | 6 (18.8) | 4 (44.4) | 4 (15.4) | 6 (66.7) |
| Antenatal depression (m, SD) | 3.34, 2.81 | 12.44, 3.32 | 3.92, 2.81 | 14.89, 4.04 |

SD: Standard Deviation, NVQ: National Vocational Qualification

Table 2 Means (standard deviations) of the salivary alpha-amylase measures

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Second trimester** | | **Third trimester** | | *F* |
| **Salivary alpha-amylase (U/ml)** | Control group (n=32) | Depressive symptom group (n=9) | Control group (n=26) | Depressive symptom group (n=9) |
| Mean awakening | 97.10 (71.87) | 112.51 (76.33) | 83.82 (60.09) | 174.39 (78.88) | 3.927\* |
| Mean awakening + 30 minutes | 63.65 (48.89) | 51.03 (38.89) | 52.49 (38.55) | 75.79 (33.72) | 0.881 |
| Mean awakening + 12 hours | 129.59 (70.00) | 118.45 (78.56) | 95.65 (53.01) | 128.70 (38.95) | 1.537 |
| log AUC | 43.14 (6.30) | 43.15 (5.81) | 41.03 (6.84) | 47.12 (2.65) | 2.238 |
| \*p<0.05 (control group *vs* depressive-symptom group (2nd and 3rd trimesters combined)) | | | | | |

## Figure legends

**Figure 1** Diurnal salivary alpha-amylase concentrations, split by group and trimester

**Figure 2** Log AUC of diurnal alpha-amylase, split by group and trimester

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